Legal Medicine 17 (2015) 9–13

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Pancreatic stone protein as a postmortem biochemical marker for the diagnosis of sepsis

Cristian Palmiere *, Marc Augsburger

University Center of Legal Medicine, Rue du Bugnon 21, 1011 Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 21 May 2014
Received in revised form 8 August 2014
Accepted 18 August 2014
Available online 26 August 2014

Keywords:
Pancreatic stone protein/regenerating protein
Acute phase proteins
C-reactive protein
Procalcitonin
Biomarkers
Inflammation markers
Inflammation
Infection

ABSTRACT

Pancreatic stone protein/regenerating protein has recently emerged as an interesting diagnostic and prognostic marker of inflammation and sepsis in the clinical field. Increased blood concentrations have been described in patients with sepsis. Moreover, a high accuracy in predicting fatal outcomes in septic patients admitted to intensive care units has been reported. In this study, we investigated pancreatic stone protein/regenerating protein in postmortem serum in a series of sepsis-related fatalities, local infections and non-infectious cases that underwent medico-legal investigations. Procalcitonin, C-reactive protein, interleukin 6, soluble triggering receptor expressed on myeloid cells-1 and pancreatic stone protein/regenerating protein were measured in the postmortem serum collected during autopsy in a group of sepsis-related deaths, local infections and non-septic intensive care unit patients. Statistically significant differences in pancreatic stone protein/regenerating protein concentrations were observed between sepsis and control patients. A significant positive correlation was found between procalcitonin and pancreatic stone protein/regenerating protein values in septic cases. Pancreatic stone protein/regenerating protein is measurable in postmortem serum from femoral blood collected during autopsy. Additionally, as in the clinical field, pancreatic stone protein/regenerating protein can be used as a postmortem biochemical marker for the diagnosis of sepsis.

1. Introduction

Early identification of patients with severe forms of infection and high risk of severe sepsis, septic shock and death continues to represent a critical step in intensive care clinical management despite significant improvements in diagnostic strategies over recent decades [1,2].

Due to the lack of specific clinical features, serum markers of inflammation/infection are commonly used to gauge decisions regarding empiric antibiotic treatment. However, owing to the limited specificity, sensitivity or both, currently measured biomarkers cannot be considered optimal. Numerous efforts have therefore been devoted to identifying not only novel sepsis parameters but also laboratory scores based on the combined determination of two or more parameters, thus attempting to overcome the limitations inherent in considering the molecules individually [1].

In forensic pathology routine, as in the clinical field, the identification of sepsis-related death is based on nonspecific features as well as inflammation and infection biomarker measurements in samples collected during autopsy. C-reactive protein (CRP), procalcitonin (PCT) and interleukin-6 (IL-6) have proven stable in postmortem samples and are routinely measured for diagnostic purposes. However, several situations of forensic interest besides bacterial infections (i.e., trauma, surgery, etc.) are responsible for increased levels of these markers. Hence, other molecules have been investigated in recent years in order to identify the most reliable combinations of biomarkers that might reliably discriminate between noninfectious and infectious inflammation [3–11].

Pancreatic stone protein/regenerating protein (PSP/reg) is a lectin-binding protein that has recently emerged as an interesting diagnostic and prognostic marker of inflammation and sepsis in both adults and newborns. Though its precise physiologic and pathologic roles are still debated, PSP/reg is assumed to behave as an acute-phase protein, regulated by cytokines produced in the damaged tissues. Increased blood PSP/reg concentrations have been described in patients with sepsis. Moreover, a high accuracy in predicting fatal outcomes in septic patients admitted to intensive care units has been reported [1,2,12–17].

In the study herein described, PSP/reg concentrations were measured in postmortem serum from femoral blood in a series of sepsis-related fatalities, local infections (pneumonia) and non-infectious cases that underwent medicolegal investigations. Our

* Corresponding author. Tel.: +41 21 314 49 61, mobile: + 41 79 556 69 89; fax: +41 21 314 70 90.
E-mail address: cristian.palmiere@chuv.ch (C. Palmiere).

http://dx.doi.org/10.1016/j.legalmed.2014.08.003
1344-6223/

aim was to determine whether PSP/reg could be reliably determined in biological samples collected during autopsy or not and assess its diagnostic potential in identifying sepsis-related deaths in the postmortem setting.

2. Materials and methods

2.1. Study design

The present study was performed during 2010–2013 and was designed as a prospective study. All cases collected for this study underwent medico-legal autopsies as requested by the inquiring authorities (the public prosecutor). Laboratory analyses, including PSP/reg measurement, were performed as part of the medico-legal investigations.

2.2. Study populations

Two study groups were prospectively formed, a sepsis-related fatalities group and a control group. The sepsis-related fatalities group consisted of 20 cases All subjects had been admitted to the intensive care unit of two academic hospitals, where they subsequently died, and had a documented, clinical diagnosis of sepsis in vivo.

According to medical records, none of these subjects suffered from acute or chronic pancreatitis, pancreatic cancer, hepatic cirrhosis, gastric cancer, chronic renal failure or diabetes mellitus at the time of hospitalization.

Sepsis was diagnosed based on evidence of pneumonia or abdominal infection along with the presence of systemic inflammatory response syndrome (SIRS) according to the definition by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) [18]. Postmortem blood samples for biochemical investigations were collected as soon as possible upon arrival of the bodies at the morgue.

Peritonitis following surgical anastomosis dehiscence, gastrointestinal perforation or intraabdominal abscess rupture (10 cases) and pneumonia (10 cases) were the infectious foci identified by means of autopsy and histology.

Sepsis and multiple organ dysfunction syndrome (MODS) were postulated as the causes of death based on autopsy, histology and biochemical analyses. PCT, CRP, IL-6 and soluble triggering receptor expressed on myeloid cells type 1 (sTREM-1) were measured in all cases. Alternative causes of death were excluded based on further postmortem investigations.

The control group consisted of 20 age-, race- and gender-matched cases. All subjects had been admitted to the intensive care unit of two academic hospitals, where they subsequently died, following trauma (motor vehicle collisions in 8 cases, falls in 6 cases and auto-pedestrians in 7 cases). All deaths occurred in the first 48 h after injury. Exsanguination and central nervous system injuries were the most frequent causes of death. None of these cases had a documented, clinical diagnosis of sepsis in vivo. No cases of lethal burns were included in this group.

According to medical records, none of these subjects had suffered from acute or chronic pancreatitis, pancreatic cancer, hepatic cirrhosis, gastric cancer, chronic renal failure or diabetes mellitus. Postmortem investigations, including microbiology, failed to reveal findings consistent with the existence of underlying acute pancreatitis or bacterial infections. Complications of undiagnosed diabetes mellitus as cause of death (diabetic ketoacidosis and hyperosmolar hyperglycemic nonketotic state) were also excluded by means of postmortem biochemistry (vitreous glucose, sodium and chloride as well as blood glycated hemoglobin, acetone and beta-hydroxybutyrate determination).

Histology, toxicology, microbiology and biochemical investigations were performed in all sepsis and control cases. Specimens for microbiological analyses were collected from at least two different sampling sites and always included cardiac blood and lung or peritoneal tissue cultures. Postmortem serum from femoral venous blood was the biological sample chosen for inflammation/infection marker measurement.

2.3. Sample collection

Peripheral blood was collected by aspiration through the femoral vein(s) prior to autopsy using a sterile needle and syringe. Venous blood was centrifuged immediately post collection at 3000*g* for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected, stored in preservative-free tubes and frozen at −20 °C until analysis. No specimens were excluded due to insufficient sample volume.

2.4. Laboratory assays

PCT, CRP, IL-6 and sTREM-1 levels were determined according to the techniques previously described [19,20]. Results were expressed in μg/L, mg/L, pg/ml and pg/ml, respectively.

The levels of PSP/reg in postmortem serum were determined using a commercialized enzyme-linked immunosorbent assay (ELISA) kit (Homo sapiens REG1a ELISA kit, USCN Life Science Inc., Houston TX, USA) according to manufacturer protocol. Results were expressed in ng/ml.

2.5. Statistical analysis

Increased postmortem serum PCT, CRP and sTREM-1 levels suggesting the presence of generalized inflammation and bacterial infections were chosen based on former medico-legal investigation results (2 μg/L, 10 mg/L, and 90 pg/ml, respectively) [5,19,20]. IL-6 cutoff value was set at 200 pg/ml, according to the findings recently reported by Llewelyn et al. [2].

PSP/reg values in postmortem serum suggesting generalized inflammation and bacterial infections were not preliminarily identified due to the unavailability of previous studies on postmortem material for comparison.

The concentration that provided the best pairing of sensitivity and specificity (1.0 ng/ml) was therefore chosen as cutoff value.

A two-group comparison was performed nonparametrically by the Mann–Whitney *U* test. *p* values less than 0.05 were considered statistically significant. Spearman's rank correlation was used to evaluate the correlation between PSP/reg and procalcitonin in septic cases. The Graphpad Prism 4.0 (Graphpad Software, La Jolla, CA, USA) was used for statistics.

2.6. Ethics

All relevant ethical issues were identified and discussed with the local Ethical Committee. All cases collected for this study underwent medicolegal autopsies as requested by the public prosecutor. Postmortem serum from femoral blood is systematically collected in our facility prior to or during autopsy and biochemical investigations are routinely performed. All postmortem serum samples were anonymized prior to analysis. No further ethical approval was necessary to perform biochemical investigations in the cases included in this study.

3. Results

Descriptive characteristics of the studied subjects are reported in Table 1. Table 2 summarizes ranges, mean values and medians